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Effects of the anesthetic steroid alphaxalone and its inactive Δ^{16}-analog on the thermotropic properties of membrane bilayers. A model for membrane perturbation

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Abstract

We have studied in detail the effects of the anesthetic steroid alphaxalone and its inactive analog Δ^{16}-alphaxalone on the thermotropic properties of model membranes using differential scanning calorimetry (DSC). The results obtained showed that, for model membranes from hydrated dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylcholine (DOPC), and egg sphingomyelin, the biologically active analog significantly broadened the phase transition, in contrast to the inactive one which produced only marginal effects. Also, alphaxalone abolished the pretransition in these preparations whereas its Δ^{16}-analog only broadened it. However, in DPPE bilayers almost no differences were observed in the effects produced by the two analogs. These results suggest that the ability of the two steroids to perturb membranes is lipid dependent. Comparisons between the effects of the two steroids on lipid/cholesterol model membranes revealed that Δ^{16}-alphaxalone excluded cholesterol from lipid/cholesterol/Δ^{16}-alphaxalone ternary systems whereas alphaxalone enhanced the effects of cholesterol and reduced the cooperativity in the binary phospholipid/cholesterol system. In an attempt to determine whether the different thermotropic effects of the two steroids on model membranes were due to (a) differences in their ability to perturb the bilayers; (b) different extents of incorporation into the bilayer, solid state ^{2}H-NMR was applied using specifically deuterated steroids. The ^{2}H-NMR data showed that alphaxalone incorporated fully into the membrane bilayer up to a molar concentration of 20%, while its inactive analog did only up to a concentration of 1%. To compare the abilities of the two steroids to perturb membrane preparations when both analogs were present in equal amounts in the membrane, the effects of very low steroid concentrations on DPPC bilayers were studied using DSC. The experiment showed that alphaxalone perturbed the membrane bilayers more effectively than its inactive analog. These results strongly suggest that the small structural differences between the two steroids are responsible for the observed differences in their abilities to perturb membranes, possibly because of differences in the packing of these two molecules within the bilayers.

Keywords: DSC; Solid state ^{2}H-NMR; NMR, solid state ^{2}H-,; Drug–membrane interaction; Steroid; Alphaxalone; Δ^{16}-Alphaxalone

1. Introduction

It is well known that only those steroid molecules that meet strict stereochemical requirements possess anesthetic activity. Alphaxalone (5α-pregnan-3α-ol-11,20-dione) has potent anesthetic properties and was used clinically as the main active component in the commercially available anesthetic Althesin. Conversely, Δ^{16}-alphaxalone (5α-pregn-16-en-3α-ol-11,20-dione), which differs from alphaxalone only by having a double bond in the C-16 position (Fig. 1), lacks anesthetic activity [1,2].

To account for their structural specificities, some investigators have suggested that anesthetic steroids act after binding to a distinct site on a target membrane protein [3] or on the $GABA_A$ receptor complex [4,5]. Alphaxalone was also found to enhance the binding of the $GABA_A$ receptor agonist muscimol to rat brain membranes at concentrations of 10^{-7} to 10^{-5} M and to potentiate the

Abbreviations: DSC, differential scanning calorimetry; DPPC, dipalmitoylphosphatidylcholine; DOPC, dioleoylphosphatidylcholine; DPPE, dipalmitoylphosphatidylethanolamine; NMR, nuclear magnetic resonance.

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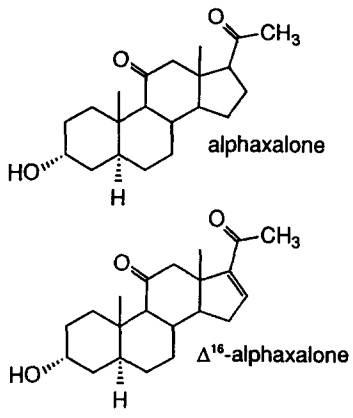

Fig. 1. Chemical structures of alphaxalone and Δ^{16}-alphaxalone.

depolarization responses to superfused GABA or muscimol on slices or rat cuneate membranes [6]. Others have hypothesized that the sites of action are the membrane lipids which are capable of a high degree of structural discrimination based on evidence obtained from electron paramagnetic resonance (EPR) experiments using spin-labeled bilayers containing cholesterol [7] and our own studies using high resolution ^{1}H-, ^{2}H- and ^{13}C-NMR [8–10]. These experiments showed that steroids possessing anesthetic activity had a fluidizing effect on model membranes while other structurally related but biologically inactive analogs produced much less disorder. O'Leary et al. [11] studied the effects of various anesthetic and non-anesthetic steroids on the DPPC bilayers using DSC and Raman spectroscopy. The DSC data showed that the steroids caused a significant depression and broadening of both the pretransition and the gel-to-liquid-crystal transition. A significant correlation was also observed between anesthetic potency and the effects of the steroids on the pretransition of hydrated DPPC while Raman spectra showed that the steroids did not perturb the head group and the glycerol backbone regions. Earlier, we studied in our laboratory the effects of alphaxalone and Δ^{16}-alphaxalone with respect to a membrane property not involved in the anesthetic response, namely, the anion transport system in human erythrocytes [12] and found that the anesthetic steroid inhibited anion transport more effectively than its inactive analog. These experiments demonstrated that the differences in biological activity between the two molecules were not restricted to their action on neuronal membranes.

Although, there are several reports in the literature regarding the effects of alphaxalone on the thermotropic properties of DPPC model membranes, the results are not always consistent with one another. Early work by Connor et al. [13] reported, without experimental details, that the incorporation of alphaxalone lowered the phase transition temperature (T_c) of DPPC bilayers by 8 K. This effect was not detected by O'Leary et al. [11], who reported a lowering of the T_c value by only 0.5 K in a hydrated DPPC preparation containing a 10% molar concentration of alphaxalone. A more detailed study in our laboratory [10] found that the incorporation of 17% molar alphaxalone in DPPC bilayers significantly affected the thermotropic properties by lowering T_c by 5 K and broadening the width of the main phase transition peak. However, the effects of alphaxalone and Δ^{16}-alphaxalone on model membranes from other lipids had not been previously examined.

In this publication, an effort was made to resolve the discrepancies in the earlier DSC results by studying the effects of alphaxalone on DPPC bilayers under different equilibration conditions. The membrane effects of the two steroids were also studied using other hydrated phospholipid preparations including DOPC, in which the lipid chains are unsaturated, egg sphingomyelin which contains a mixture of saturated and unsaturated fatty acid species and DPPE in which the headgroup is ethanolamine instead of choline. We also used cholesterol-containing preparations to obtain lipid/steroid/cholesterol ternary systems.

To measure the extent of incorporation of alphaxalone and Δ^{16}-alphaxalone in DPPC bilayers we used solid state ^{2}H-NMR spectroscopy. The results allowed us to more carefully evaluate the factors which determine the observed differences between the two steroids with regard to their abilities to perturb membrane bilayers. They also allowed us to distinguish between those effects which can be attributed to differences in the amounts of steroid incorporated into the bilayer from those due to the steroid's conformational properties [9].

2. Materials and methods

2.1. Materials

DPPC, DOPC, DPPE and egg sphingomyelin were obtained from Avanti Polar Lipids, Birmingham, AL. According to the chromatographic data provided by the company, egg sphingomyelin was a mixture of several species containing saturated (14:0, 16:0, 18:0, 20:0, 22:0, 24:0) and unsaturated (18:1, 22:1, 24:1) acyl residues, the major component being the palmitoyl residue which constituted 77% of the mixture. Alphaxalone and Δ^{16}-alphaxalone were kindly donated by Glaxo Group Research, Middlesex, UK.

2.2. Methods

Sample preparation procedures were identical for DSC and solid state ^{2}H-NMR experiments. Appropriate amounts of phospholipid and protonated or deuterated steroids (with or without cholesterol) were dissolved in spectroscopic grade chloroform. The solvent was then evaporated by passing a stream of O_2-free nitrogen over the solution at 50°C and the residue was placed under vacuum (0.1 mmHg) for 12 h.

For DSC experiments, distilled and deionized water was added to the dried mixtures (ca. 5 mg) to produce a 50% (w/w) lipid/water preparation. The sample was then placed in a stainless steel capsule (Perkin-Elmer) and sealed hermetically and thermograms were obtained on Perkin-Elmer DSC-2 and DSC-7 instruments. The calorimeter temperature scale was calibrated using standard samples such as fully hydrated DPPC and pure crystalline indium. To ensure complete equilibration, the sample was held at a temperature above its phase transition temperature for 5 min prior to scanning. Furthermore, each sample was scanned at least twice until identical thermograms were obtained. We found that the optimal scanning rate is 2.5 K/min, as higher or lower scanning rates produced some distortions in the thermograms. Being aware that the method of sample preparation can significantly affect the appearance of the thermogram, we used for our comparisons thermograms from samples prepared in an identical manner and having an identical thermal history.

For the solid state ^{2}H-NMR, the dried mixture (ca. 150 mg) was introduced into a 7 mm glass tube and hydrated using deuterium-depleted water to produce a 50% (w/w) lipid-water preparation, cooled in liquid nitrogen and sealed under high vacuum. Solid state ^{2}H-NMR spectra were obtained (at 55.2 MHz) using a 90°_x-τ-90°_y quadrupole echo sequence [14] with a 90° pulse width of 2.1 to 2.4 μs, a pulse separation (τ) of 40 μs and a dwell time of 3.5 μs. Recycle delay was 0.2 s, and up to 16000 echoes were accumulated for each spectrum.

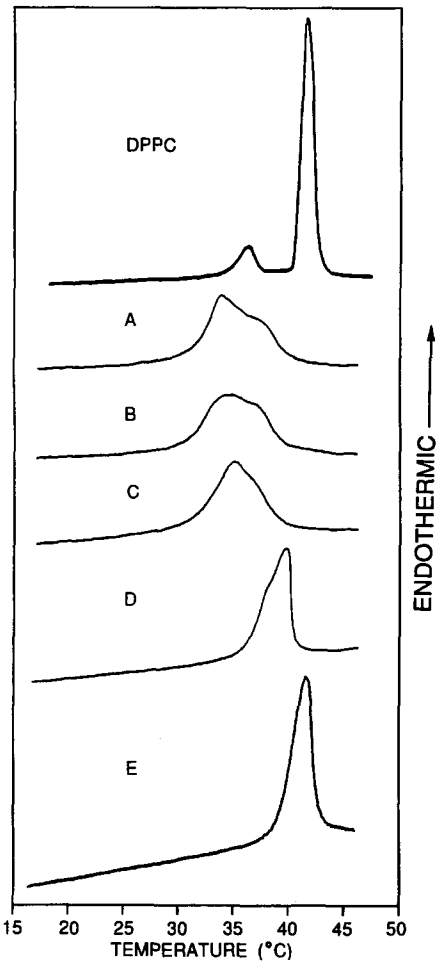

Fig. 2. Normalized thermogram of DPPC bilayer without steroid and thermograms of DPPC bilayers containing alphaxalone ($x = 0.20$) obtained (A) right after sealing; (B) second run; (C) third run; (D) after leaving at room temperature for 5 days; (E) after leaving in the freezer for 5 additional days.

3. Results and discussion

3.1. Effect of equilibration conditions on the thermotropic properties of DPPC + alphaxalone

Our first goal was to reconcile the apparent inconsistencies in the thermotropic data of different laboratories obtained from DPPC bilayer samples containing alphaxalone and we hypothesized that this was due to differences in the thermal histories of the samples. To explore this possibility we subjected a DPPC/alphaxalone preparation with a molar ratio of 80:20 ($x = 0.20$) to different equilibration conditions, before performing a series of DSC experiments. Fig. 2 shows a thermogram from a pure DPPC bilayer preparation and five thermograms from a DPPC/alphaxalone preparation run at different stages of equilibration. When the sample was run immediately following hydration, the phase transition temperature was about 5 to 8 K below that of DPPC ($T_c = 41.2$°C). For example, T_c was 33°C in the first run and increased to 35°C by the third run. Other subsequent scans gave identical thermograms to that obtained in the third run. These results were in agreement with the results obtained by Connor et al. [13] and ourselves [10] where it was reported that the presence of alphaxalone in DPPC bilayers reduced the phase transition temperature by 8 K and 4–5 K, respectively. When the sample was kept at room temperature for 5 days the thermogram had a T_c at 40°C, only about 1 K below that of pure DPPC. We also found that when the sample was left in the freezer for an additional 5 days it showed a T_c of 41°C, perhaps explaining the results reported earlier by O'Leary et al. [11].

As can be seen in Fig. 2, the lineshapes of the thermogram vary depending on equilibration time and temperature. Generally, the observed broadening of the principal phase transition produced by alphaxalone diminished with longer equilibration times, while the enthalpy change (ΔH) remained constant throughout the thermal history.

3.2. Effects of the two steroids on the thermotropic properties of model membranes. Lipid dependent variations of DPPC, DOPC, sphingomyelin and DPPE

To gain a better understanding on the specificity of the steroid–membrane interactions, we studied the effects of

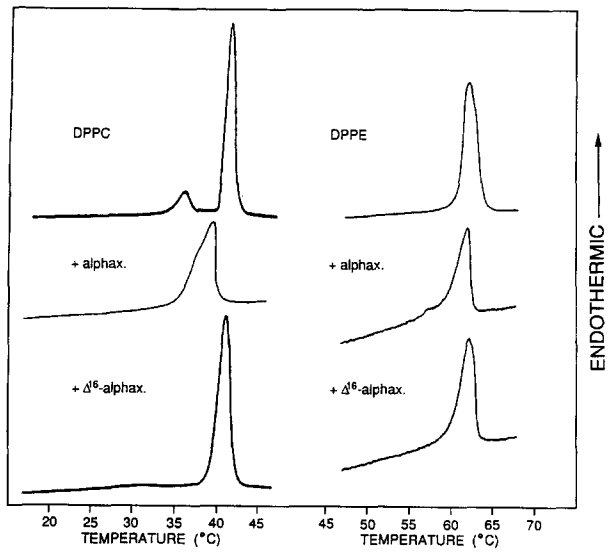

Fig. 3. Normalized thermograms of model membrane preparations of DPPC (left traces) and DPPE (right traces). Top traces: lipid bilayer alone; middle traces: containing alphaxalone ($x = 0.20$); bottom traces: containing Δ^{16}-alphaxalone ($x = 0.20$).

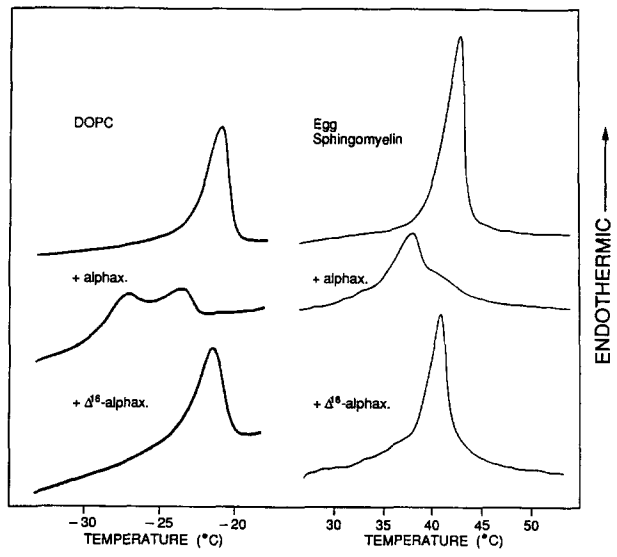

Fig. 4. Normalized thermograms of model membrane preparations of DOPC (left traces) and egg sphingomyelin (right traces). Top traces: lipid bilayer alone; middle traces: containing alphaxalone; bottom traces: containing Δ^{16}-alphaxalone. Steroid concentrations: $x = 0.20$ in DOPC preparations and $x = 0.10$ in egg sphingomyelin preparations.

the two steroids on the thermotropic properties of model membranes prepared from different phospholipids using DSC (Figs. 3 and 4). We found that all membranes prepared from phospholipids possessing choline headgroups were affected very differently by each of the two steroids. However, neither steroid had any significant effect on hydrated DPPE preparations.

In the DPPC preparation, alphaxalone ($x = 0.20$) produced a significant broadening of the main transition which seemed to be composed of two overlapping components while the pretransition was no longer discernible (Fig. 3, left). This broadening of the main transition was interpreted as decreased cooperativity at the phase transition. Conversely, the inactive Δ^{16}-alphaxalone did not affect the half width and T_c of the main transition of the hydrated DPPC preparation; only the pretransition was broadened and appeared at a lower temperature.

The effects of the two steroids on DOPC bilayers were strikingly different (Fig. 4, left). Alphaxalone produced a dramatic broadening of the phase transition as manifested by the appearance of two transition maxima, a phenomenon which may be attributed to a phase inhomogeneity in the model membranes caused by the presence of the steroid [14,15]. This may, in turn, reflect the existence of two domains each represented by one peak in the thermogram. Thus, the peak on the right may be due to a DOPC domain containing a low concentration of alphaxalone while the peak on the left may represent a DOPC domain with a high concentration of the steroid. Δ^{16}-Alphaxalone had almost no effect on the thermograms of DOPC bilayers. Distinct differences were also observed between the effects of the two analogs on egg sphingomyelin preparations (Fig. 4, right). Alphaxalone lowered the phase transition temperature and broadened it significantly, whereas Δ^{16}-alphaxalone once again produced almost no effect.

Unlike the membrane preparations from phospholipids with choline headgroups, DPPE preparations were very marginally affected by the presence of each of the two steroids giving almost identical thermograms with that from the pure lipid and showing no changes in their transition temperatures and peak widths. This may be the result of very limited or no incorporation (Fig. 3, right) of the steroids into hydrated DPPE.

It is evident from the above experiments that the effects of the two steroids on model membranes are highly dependent on the nature of the phospholipid used in the preparation. Although it is difficult to fully explain these differences using molecular arguments, some correlation seems to exist between the thermotropic effects induced by the steroid on the membrane and the extent of phospholipid hydration; the more hydrated the phospholipid the more susceptible it is to perturbation by the steroids. Thus, the most hydrated unsaturated choline phospholipid (DOPC) experiences the greatest perturbation by alphaxalone followed by the less hydrated DPPC [16–18]. DPPE is the least hydrated [19] of the phospholipids used in our study and is only marginally affected by the steroids.

3.3. DPPC, DOPC and DPPE bilayers containing cholesterol

Fig. 5 depicts the effects of the two steroids on DPPC model membranes containing two different molar ratios of cholesterol $x = 0.08$ (left) and $x = 0.20$ (right). The thermogram from the $x = 0.08$ cholesterol preparation shows a

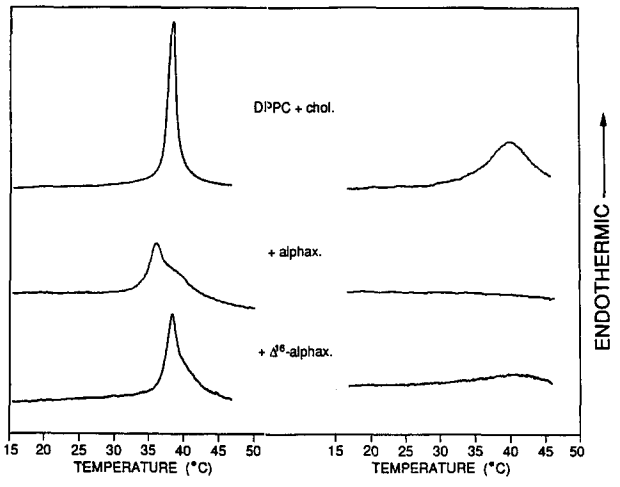

Fig. 5. Normalized thermograms of model membrane preparations of DPPC + cholesterol with cholesterol concentrations of $x = 0.08$ (left traces) and $x = 0.20$ (right traces). Top traces: lipid + cholesterol bilayer alone; middle traces: with additional alphaxalone ($x = 0.20$); bottom traces: with additional Δ^{16}-alphaxalone ($x = 0.20$).

sharp peak centered at 38.5°C. When the cholesterol concentration is increased to $x = 0.20$, this peak is now broadened and has a smaller area indicating that the enthalpy change associated with the gel to liquid crystal phase transition is reduced. These results are consistent with a previous detailed study related to the thermotropic behaviour of the DPPC/cholesterol bilayers in which cholesterol [20] was shown to reduce the cooperativity between the lipids in the membrane, thus, serving as a buffer to temperature changes near the phase transition.

The addition of alphaxalone ($x = 0.20$) to the DPPC + cholesterol ($x = 0.08$) preparation transforms the sharp component of the thermogram into a broad phase transition while a new sharper component appears around $T_c = 36$°C. Conversely, Δ^{16}-alphaxalone causes less significant broadening of the transition and does not lower the phase transition temperature. Addition of alphaxalone to the DPPC preparation with the higher cholesterol content ($x = 0.20$) almost completely eliminates the endotherm peak associated with the phase transition, a reflection of the severe effect it has on chain cooperativity. The effects of Δ^{16}-alphaxalone on the same preparation are much less striking and involve only broadening of the endotherm but no change in the phase transition temperature.

Fig. 6 depicts the thermotropic effects of the two steroids on the DOPC/cholesterol ($x = 0.20$) preparation which, in the absence of drug, shows a broad phase transition $T_c = -26.8$°C. Addition of alphaxalone ($x = 0.20$) to this preparation broadens this transition which now appears as an endotherm with two peaks, suggesting the existence of two domains. The peak on the right has the same T_c as the parent preparation and probably represents a DOPC/cholesterol domain, while the peak on the left is due to another domain composed of DOPC, cholesterol

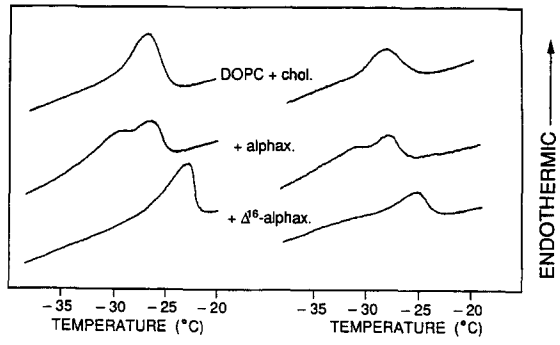

Fig. 6. Normalized thermograms of model membrane preparations of DOPC + cholesterol with cholesterol concentrations of $x = 0.20$ (left traces) and $x = 0.35$ (right traces). Top traces: lipid + cholesterol bilayer alone; middle traces: with additional alphaxalone ($x = 0.20$); bottom traces: with additional Δ^{16}-alphaxalone ($x = 0.20$).

and alphaxalone. Again Δ^{16}-alphaxalone has a different effect on this membrane preparation. However, unlike the marginal changes produced by this steroid on the thermograms of other membrane preparations, we now observe an upward shift of about 4 K in the T_c. A closer examination of the DOPC/cholesterol/Δ^{16}-alphaxalone thermogram reveals that it resembles the one obtained from the pure DOPC preparation (Fig. 4). This leads us to the conclusion that the steroid excludes much of the cholesterol from the bilayers by complexing with it and forming a different domain which does not significantly affect the thermotropic properties of the hydrated DOPC system. Similar results were obtained with preparations containing higher concentrations of cholesterol ($x = 0.35$) (Fig. 6). These results are reminiscent of earlier data [21] with DMPC preparations in which we first postulated the formation of a Δ^{16}-alphaxalone-cholesterol complex.

Fig. 7 shows the effects of the two steroids on DPPE/cholesterol ($x = 0.20$) preparations. The thermo-

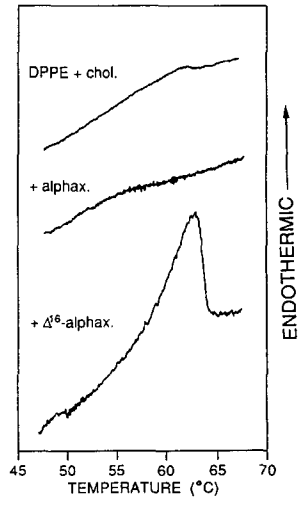

Fig. 7. Normalized thermograms of hydrated (A) DPPE + cholesterol $x = 0.20$ (top trace), (B) with additional alphaxalone $x = 0.20$ (middle trace) and (C) with additional Δ^{16}-alphaxalone $x = 0.20$ (bottom trace).

grams appear to follow a similar trend as those from the other cholesterol containing samples. The initial sample has a very broad phase transition which is further broadened and shifted to a lower temperature by the addition of alphaxalone. Conversely, the biologically inactive Δ^{16}-analog significantly sharpens the phase transition and shifts it to a higher temperature. The thermogram now resembles the one obtained from a DPPE preparation without cholesterol (Fig. 3) and again suggests displacement of cholesterol from the bilayer through the formation of a Δ^{16}-alphaxalone-cholesterol complex.

Interestingly, we find that Δ^{16}-alphaxalone displaces cholesterol more effectively in hydrated phosphatidylethanolamine than phosphatidylcholine preparations. This trend is not observed with alphaxalone which produces analogous effects on the different cholesterol-containing bilayers as those observed with preparations containing no cholesterol.

3.4. The molecular mechanism of bilayer perturbation by anesthetic steroids

The observed differences between alphaxalone and Δ^{16}-alphaxalone in their abilities to affect the thermotropic properties of model membranes can be attributed to one of two causes or a combination of the two. The first of these is the difference in the amounts of each of the two steroids in the bilayer with alphaxalone appearing to incorporate more effectively than its Δ^{16}-congener. The second reason is associated with the steroid's ability to pack properly within the membrane bilayers, a property which is highly sensitive to the stereoelectronic features of this molecule. As we proposed in earlier publications [8,9], imperfect packing of the lipid chains is associated with conformational and dynamic changes in the chain segments. These effects, which are observed as characteristic changes in the thermograms of the bilayer preparation, can be described generically as 'membrane perturbation'. A good example of a membrane-perturbing steroid is alphaxalone whose D-ring protrudes from the plane of the other three. This results in imperfect packing within a variety of phospholipid bilayers. Conversely, the biologically inactive, Δ^{16}-alphaxalone has all four rings coplanar, packs better and produces no significant perturbation. To dissect these two factors which may be responsible for membrane perturbation by steroids or other membrane-active molecules, namely, (a) the degree of incorporation and (b) the packing characteristics of the ligands in the bilayer, we used a combination of solid-state ^{2}H-NMR and DSC.

3.5. Extent of incorporation of the steroids in DPPC bilayers

Solid-state ^{2}H-NMR was used as the method of choice for obtaining direct information on the amount of the steroid present within the bilayer. In such an experiment

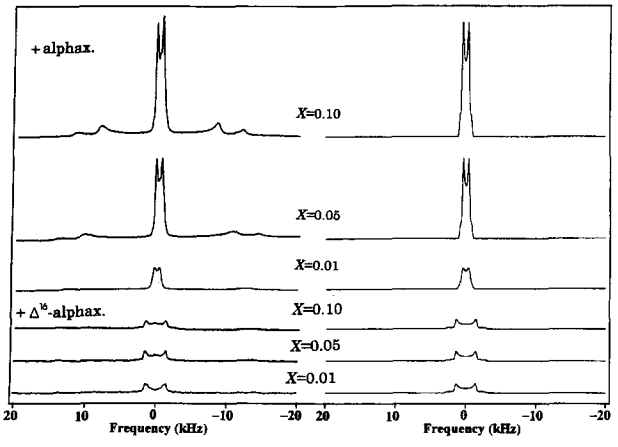

Fig. 8. Solid state ^{2}H-NMR spectra from hydrated DPPC bilayer preparations containing (9,12α,12β,17,21,21,21-d_7)-alphaxalone or (9,12α,12β,21,21,21-d_6)-Δ^{16}-alphaxalone ($x = 0.01$, 0.05 and 0.10). Left: experimental data. Right: simulated spectra.

which is conducted on a stationary sample, the spectrum due to the ^{2}H-labeled steroid is expected to appear either as a relatively narrow singlet if the steroid is present in solution undergoing isotropic motions or as a characteristic Pake pattern, if the steroid is embedded in the anisotropic environment of the bilayer [22].

Fig. 8 on the left, depicts typical solid state ^{2}H-NMR spectra of DPPC bilayer preparations containing deuterium-labelled alphaxalone and Δ^{16}-alphaxalone at 42°C, each in three different concentrations ($x = 0.10$, 0.05, and 0.01). To standardize our measurements, we used the same amount of DPPC in each of the samples. Furthermore, the spectra were obtained using identical experimental parameters, thus allowing us to correlate spectral intensities with the amount of drug incorporated in the bilayer. Because of their very limited solubility in water, the steroids do not exist in any significant amount dissolved in the aqueous phase of the membrane preparation. Therefore, they are expected either to intercalate between the chains of the hydrated phospholipid or, alternatively, to form solid aggregates which segregate outside of the bilayer. Under the experimental conditions used, the observed spectrum is solely due to the population of steroid molecules which is incorporated in the bilayer, since those molecules present in the solid phase do not significantly contribute to the ^{2}H-spectrum.

In all of the spectra, the central doublet is due to the -$COCD_3$ group in the 17-position and is used as a measure of incorporation of each of the two steroids. The spectra from the alphaxalone-containing preparations also include two wider and less intense doublets due to other deuterons on the steroid ring which have only partially exchanged with the respective protons.

We are able to quantitatively evaluate the spectral areas under each of the narrow doublets using spectral simulations as can be seen on the right in Fig. 8. A more expanded solid-state ^{2}H-NMR study which will include a

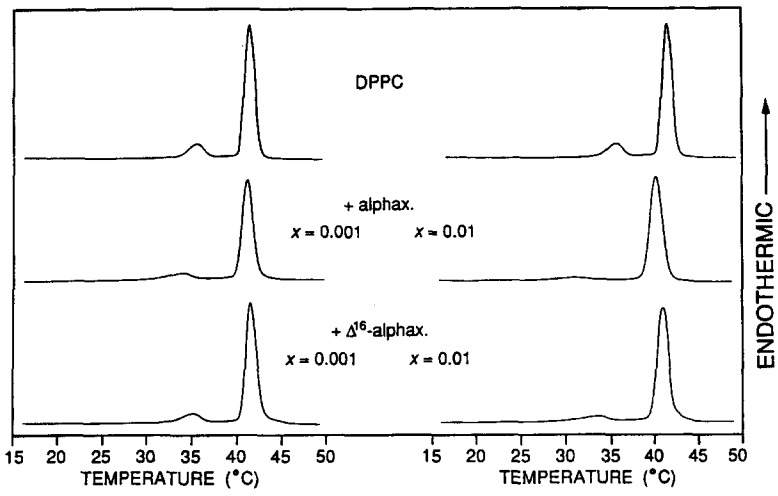

Fig. 9. Normalized thermograms of model membrane preparations of DPPC containing steroids with low concentrations of $x = 0.001$ (left traces) and $x = 0.01$ (right traces). Top traces: lipid bilayer alone; middle traces: DPPC + alphaxalone; bottom traces: DPPC + Δ^{16}-alphaxalone.

large number of steroid molecules will be described elsewhere and will give a more detailed account of the methods and experimental conditions used in this experiment.

The ^{2}H-spectra from the inactive Δ^{16}-analog show a much less intense narrow doublet, an indication of poor incorporation of the steroid in the bilayer. All three spectra are of nearly equal intensity indicating that at a concentration of $x = 0.01$ saturation had already occurred. Additionally, the spectral quantitation allowed us to estimate that at a steroid concentration of $x = 0.01$ both steroids incorporated nearly equally.

3.6. Effects of low steroid concentrations on hydrated DPPC bilayers

Having determined by an independent method the concentration limit below which both steroids incorporate equally into the DPPC bilayers, we were now able to design an experiment through which the steroid's ability to perturb the model membrane could be studied and correlated with its molecular features and its packing properties in the bilayer. The experiment involved the use of DSC to observe the thermotropic properties of DPPC bilayer preparations containing low steroid concentrations ($\leq$ 0.01).

Fig. 9 depicts two sets of thermograms corresponding to preparations with $x = 0.01$ and $x = 0.001$ steroid concentrations. Changes in the main transition are not easily discernible at the low steroid concentrations. However, such differences can be observed clearly at the pretransition endotherm of the different DPPC preparations.

At the higher steroid concentration of $x = 0.01$ the differences between the thermal pretransitions of the two preparations are even starker with the alphaxalone-containing preparation having a considerably broader pretransition.

The above experiment demonstrates that when incorporated equally in a membrane system, the two steroids exert very different effects on the conformational and dynamic properties of the bilayer. The data clearly indicate that alphaxalone perturbs the membrane bilayer system more effectively than its inactive Δ^{16}-analog. This observation lends support to the hypothesis according to which the ability of membrane-active compounds to perturb membranes is closely linked to imperfect packing of these molecules in the bilayer.

4. Conclusions

(1) The anesthetic steroid alphaxalone affects the thermotropic properties of membrane preparations from DPPC, DOPC and sphingomyelin more effectively than its biologically inactive analog Δ^{16}-alphaxalone. However, both steroids do not significantly perturb hydrated DPPE preparations. Some correlation may exist between the extent of hydration of the phospholipid polar group and the degree to which the membrane preparation is affected by membrane-active steroids.

(2) Alphaxalone incorporates relatively easily within membrane-bilayers. However, the degree of incorporation can be greatly affected by the equilibration conditions. Conversely, Δ^{16}-alphaxalone incorporates in membrane bilayers sparingly, a property which may be related to the low solubility of this steroid in organic solvents presumably because of tight intermolecular packing in its solid state.

(3) At concentrations which are low enough to allow both steroids to be fully incorporated in DPPC model membranes, alphaxalone perturbs the bilayers more effectively than Δ^{16}-alphaxalone. This difference can be explained by invoking an imperfect packing of alphaxalone

within the membrane resulting in changes in the conformational and dynamic properties of the bilayer chains. Conversely, Δ^{16}-alphaxalone does not significantly affect the packing of the lipid chains in the bilayer and perturbs the membrane minimally.

(4) Alphaxalone perturbs membranes more effectively than its Δ^{16}-congener because of (a) better incorporation into the bilayer (b) its ability to induce conformational and dynamic changes in the bilayer chains. We can postulate that perturbation of the membrane lipids by alphaxalone may affect the functional properties of one or more membrane-associated proteins and explain its anesthetic properties.

(5) In cholesterol-containing membrane bilayers Δ^{16}-alphaxalone forms cholesterol/Δ^{16}-alphaxalone complexes thus, effectively reducing the concentration of cholesterol within the bilayer. Alphaxalone does not form such complexes but reduces the cooperativity during the phase transition in the phospholipid/cholesterol system. It is unclear whether the above differences in how the two steroids interact with phospholipid/cholesterol mixtures are relevant to their physiological properties.

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